**A simple and sensitive electrochemical sensor for new neonicotinoid
insecticide Paichongding in grain samples based on
 β -cyclodextrin-graphene modified glassy carbon electrode**

M. Zhang^{a,b,#}, H. T. Zhao^{a,#}, X. Yang^{a,b,*}, A. J. Dong^a, H. Zhang^a, J. Wang^{a,b,*}, G. Y. Liu^a, X. C. Zhai^a

^aDepartment of Food Sciences and Engineering, School of Chemical Engineering and Technology,
Harbin Institute of Technology, 150090 Harbin, PR China

^bKey Laboratory of Agro-Product Quality and Safety, Institute of Quality Standard and Testing
Technology for Agro-Product, Chinese Academy of Agricultural Sciences, 100081 Beijing, PR China

[#]These authors contributed equally to this work and should be considered as co-first authors.

***Corresponding author:**

Prof. X. Yang

Department of Food Sciences and Engineering, School of Chemical Engineering and Technology,

Harbin Institute of Technology

No.73 Huanghe Road, Nangang District, Harbin, Heilongjiang Province P. R. of China 150090

Tel: +86451-86282910, Fax: +86451-86282906, E-mail: yangxin@hit.edu.cn

Prof. J. Wang

Key Laboratory of Agro-product Quality and Safety, Institute of Quality Standard & Testing

Technology for Agro-Product, Chinese Academy of Agricultural Sciences

No.12 South Street, Zhongguancun, Haidian District, Beijing, Beijing Province P. R. of China 100081

Co-authors:

M. Zhang, H. T. Zhao, A. J. Dong, H. Zhang, G. Y. Liu, X. C. Zhai

Department of Food Sciences and Engineering, School of Chemical Engineering and Technology,

Harbin Institute of Technology

No.73 Huanghe Road, Nangang District, Harbin, Heilongjiang Province P. R. of China 150090

Abstract: A simple and sensitive sensor based on β -cyclodextrin-graphene modified glassy carbon electrode (β -CD-rGO/GCE) was developed for the electrochemical determination of new neonicotinoid insecticide Paichongding (IPP). In 0.1 M phosphate buffer solution, the electrode offers low reduction potential and the peak current of IPP increased significantly at β -CD-rGO/GCE compared with bare GCE and rGO/GCE. Furthermore, low temperature is beneficial to further improve the peak current and determination sensitivity. The reduction mechanism and binding affinity were discussed. The experimental conditions that exert influence on the voltammetric determination of IPP, such as temperature, pH values and accumulation time were optimized. Besides, the interference, reproducibility and stability measurements were also evaluated. Under the optimal experimental conditions, a linear relationship between the peak current and the concentration was obtained from linear sweep voltammetry in the range from 1 to 10 μ M, 10 to 55 μ M, with the detection limit of 0.11 μ M and quantification limit of 0.37 μ M. This sensor was used successfully for IPP determination in grain samples. The results show good agreement with the HPLC method.

Keywords: Neonicotinoid insecticide; Paichongding (IPP); Electrochemical sensor; β -cyclodextrin; graphene; Low temperature

1 Introduction

The discovery of neonicotinoids can be considered as a milestone in agrochemical research over the past three decades. This insecticide has been one of the world's largest selling insecticide (24% of the total world market) in more than 120 countries because of excellent effectiveness and relatively low toxicity to mammals as well as the environment when compared to organophosphates and carbamates [1-2]. The neuro-active insecticide, 1-[6-chloro-3-methyl-pyridyl-8-nitro-7-methyl-1,2,3,5,6,7-hexahydro imidazo- (1,2a)]-pyridine (Paichongding, IPP), is a new third-generation neonicotinoid pesticide, developed by the East China University of Science and Technology, Shanghai, PR China. This insecticide focuses on the protection of Homopteran pest; an international patent was granted in 2004. The selective nature of neonicotinoids plays a central role on its safety to mammals as they are used for the control of sucking insect pests [3]. Due to extensive usage, high water solubility (1.46 g/L at 25°C) and stability, the residues of the IPP persistent in the environment produce adverse effects.

The Positive List System for Agricultural Chemical Residues in Food in Japan has set the maximum residue limits (MRLs) for neonicotinoid insecticides in different agricultural products, including vegetables and grains. The MRLs were between 0.01 and 1.50 mg kg⁻¹ [4]. Accordingly, there is a growing need to develop a rapid and simple method for the characterization and determination of neonicotinoids present in food. The existing analytical techniques for the analysis of neonicotinoid including IPP are high-performance liquid chromatography (HPLC) [5], thermal lens spectrometric detection [6] and immunoassay methods [7]. All these methods require sophisticated instruments and highly skilled personnel for operation and produce the toxic organic solvents as waste and disposal of these toxic organic solvents which is again a serious

environmental concern [8]. As is well known, modern electrochemistry represent a convenient, fast and inexpensive alternative method which are suitable for a large-scale monitoring of different electrochemically active environmental pollutants and easily adaptable for in-the-field work. Our group have already established a multi-residue method for determination of seven neonicotinoid insecticides in grains using dispersive solid-phase extraction and dispersive liquid-liquid micro-extraction by HPLC [9] and evaluated effect of IPP on wheat plant's antioxidant defense system [10]. In continuation of our effort to develop the analysis of IPP, we report the convenient, fast, inexpensive and sensitive electrochemical sensing of IPP in this paper.

Voltammetric determination of neonicotinoids such as imidacloprid and thiamethoxam on glassy carbon electrode has been reported with the detection limit of 29 μM . The reported detection limit is too high to carry out the trace level analysis. The reduction peak was observed at around -1.3 V which is intimate to the hydrogen evolution region [11]. Determination of neonicotinoid insecticides using differently modified glassy carbon electrodes such as Nanosilver Nafion/nanoTiO₂ Nafion composite, silver amalgam film and nitrogen-doped graphene has been reported [12-14]. There was few analytical data reported for the electrochemical determination of IPP in these papers. Besides, it is apparent that metal nanoparticles are the main solution to improve the determination sensitivity, nevertheless, they are expensive. Due to the large surface area, high conductivity, high supramolecular recognition and enrichment capability properties, functionalized graphene including β -cyclodextrin functionalized graphene (β -CD-rGO) modified electrodes attract the attention of researchers in greater extent. It has been applied in chiral recognition of chlorophenol [15] and tryptophan [16], as well as determination of methionine [17], isoquercitrin, baicalin [18], cholesterol [19]. In order to significantly improve the detection

sensitivity, nanotube [20], cytochrome c [21] and Au nanoparticles [22] have been applied to enhance the electrochemical signals via synergistic effect. In this paper, we found that low temperature is a simple solution to further improve the peak current and determination sensitivity of IPP on β -CD-rGO modified glassy carbon electrode (β -CD-rGO/GCE). Cyclic voltammetry and Linear sweep voltammetry were used as sensing techniques. The results obtained in electrochemical techniques are compared with the HPLC method.

2 Experimental

2.1 Instruments and reagents

Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) were performed on a CHI 660E electrochemical workstation (Chenhua Instrument Co., Ltd., Shanghai, China). A conventional three-electrode system was adopted. A glassy carbon electrode (GCE, $\varnothing$ 3mm) was used as the working electrode, a platinum wire was used as the auxiliary electrode and Ag/AgCl (3 M KCl) electrode was used as the reference electrode. An Agilent Technology 1100 high performance liquid chromatography (HPLC) connected to a diode array detector was employed. In addition, an Agilent-C₁₈ column (4.6 $\times$ 250 mm i.e., 5.0 μ m) was used.

Graphite powder, hydrazine hydrate solution (80 wt%), KMnO₄, H₂SO₄, HCl, KNO₃ and β -cyclodextrin were all of analytical grade and obtained from Beijing Chemical Reagents Company. The IPP was purchased from the Kwin Company Ltd. Nantong, Jiangsu Province PR China. A standard stock solution of 1.0×10^{-3} M IPP was preserved at 4 °C and protected from daylight when not in use. Working solutions of lower concentrations were prepared by appropriate dilution of the stock solution with 0.1 M phosphate buffer solution (PBS) which was used as the supporting electrolyte. All other reagents were Chromatography analytical grade and prepared

with ultrapure water (18.2 MΩ cm) from a [Millipore Milli-Q](#) system. Standard solution of IPP were prepared in PBS.

2.2 Fabrication of β -CD/rGO modified electrodes (β -CD/rGO/GCE)

Graphene oxide (GO) was synthesized from graphite powder by the modified Hummers method [23] and β -cyclodextrin/graphene (β -CD/rGO) was prepared by the reported method [24]. Prior to modification, the GCE was polished with 1, 0.3, and 0.5 μ m alumina slurry, rinsed thoroughly with doubly distilled water between each polishing step, then washed successively with 1:1 nitric acid, acetone, and doubly distilled water in an ultrasonic bath and dried in air. The β -CD-rGO/GCE, rGO/GCE were obtained by casting 5 μ L of 0.25 mg mL⁻¹ suspension on the surface of a well-polished GCE and dried in air. Finally, the modified electrodes were activated by several successive scans with a scan rate of 50 mV s⁻¹ in phosphate buffer solution (pH 7.2) until a steady voltammogram was obtained.

2.3 Electrochemical analysis

For voltammetric measurements, the three-electrode system was immersed in a 10-mL electrochemical cell containing an appropriate concentration of IPP and 0.1 M PBS solution. Following the pre-concentration period, cyclic voltammograms or linear sweep voltammograms were recorded at a scan rate of 0.05 V s⁻¹. The potential scan ranges were -0.2 ~ -1.4V for cyclic voltammograms and -0.2~ -1.2 V for the linear sweep voltammograms. The electrochemical impedance spectroscopy was scanned in 5mM Fe(CN)₆^{3-/4-} and 0.1M KCl solution at the formal potential of 0.25V with a frequency range of 0.01~10⁵ Hz and a signal amplitude of 5 mV. The same procedure was carried out in the sample analysis and the result was compared with HPLC analysis method of IPP developed in our laboratory. Besides, the pretreatment of the commercial

food samples was also performed as the our group's previous work [9].

2.4 Method performance

The sensitivity and precision of the method were evaluated by spiked blank grain samples. Recoveries and RSD were determined for two replicates at four concentration levels (1.6, 6.4, 8.0, 10 μ M) under the detection condition of 0 $^{\circ}$ C and two concentration levels (10 and 32 μ M) under the detection condition of 25 $^{\circ}$ C. The limit of detection (LODs) was determined by considering a signal-to-noise ratio of 3 with reference to the background noise obtained from blank solution whereas the limits of quantification (LOQs) were determined by considering as signal-to-noise ratio of 10.

3 Results and discussion

3.1 The protocol of the sensor

Schematic procedure for the preparation of β -CD-rGO/GCE and our concept for enhancing the sensitivity and specificity of electrochemical IPP detection are shown in Scheme 1 . Firstly, a simple wet-chemical route is employed for synthesizing β -CD-rGO, initially when graphite is treated in the presence of strong oxidizing agent which provides oxygen functionalities (mostly epoxy, hydroxyl, carbonyl and carboxyl groups) to make GO soluble in water [18]. Since β -CD and rGO contain multiple OH groups, the formation of strong hydrogen bonding between them is strongly supported. Then, the strong reducing agent hydrazine consisting mostly of oxygen-containing groups would be reduced to produce the rGO [24]. β -CD not only serves as stabilizer for the rGO but also as a supramolecular recognition element to enhance the sensitivity of detection through the formation of supramolecular complexes between β -CD and the guest molecule IPP. After preparation of β -CD-rGO, the modified GCE was fabricated by dropping 5 μ L

β -CD-rGO solution on the electrode surface, dried in air and activated in PBS. The pre-concentration period is necessary for high electrochemical response and low temperature is applied to further improve the peak current and determination sensitivity of IPP on β -CD-rGO/GCE.

3.2 Electrochemical behavior of IPP on the GCEs

The electrochemical reduction of IPP on different modified GCEs by CV were carried out in 0.1 M PBS (pH 7.2) containing 50 μ M IPP in order to make a comparison of the electrochemical properties, as shown in Fig. 1 A. A small reduction wave around -1.2V was observed on bare glassy carbon electrode (curve a). There is no reverse peak obtained for all the cyclic voltammetric scans which shows that IPP undergoes irreversible reduction on β -CD-rGO modified and bare glassy carbon electrodes. Compared with the bare GCE, about 14-fold larger current signal was obtained at around -1.0 V on β -CD-rGO/GCE, which demonstrated that the β -CD-rGO/GCE could greatly facilitate the reduction of IPP. The rGO displayed the excellent conductivity and a large surface area which provided more active sites and enhanced the transfer of charge between the electrode and the reagent. Meanwhile, β -CD improved the uniform distribution, catalytic activity and durability of catalysts which can be demonstrated from the narrow peak of IPP on β -CD-rGO/GCE in comparison with rGO/GCE [25].

To investigate the reaction mechanism, CV experiments were carried out at β -CD-rGO/GCE to realize how the scan rate affected the reduction of 50 μ M IPP in 0.1M PBS (pH 7.2). The results are shown in Fig. 1B. According to Nicholson equation (Eq. (1))[26], the cathodic peak potential E_p for an irreversible cathodic reaction can be described as following:

$$E_p = E^{0'} + \frac{RT}{\alpha n_a F} \left[0.781 + \ln \left(\frac{D_R^{1/2}}{k^0} \right) + \ln \left(\frac{\alpha n_a F v}{RT} \right)^{1/2} \right] \quad (1)$$

Where $E^{0'}$ is the formal standard potential, α is the electron transfer coefficient, n_a is the number of the electrons transferred in the rate determining step, F is the Faraday constant, R is the gas constant, T is the absolute temperature. A linear relationship between E_p and Napierian logarithm of v ($\ln v$) is observed in the range of 10-100 mV s^{-1} . The equation can be expressed as $E_p (\text{V}) = -0.047 \ln v - 0.884$ ($R=0.997$) (Fig. 1D). The slope of the line is equal to $RT/2\alpha n_a F$. Therefore, αn is calculated to be 0.28. Generally, for a totally irreversible electrode process, α is between 0.3 to 0.7, so the electron transfer number (n) involved in the reduction of IPP is around 1 ($\alpha = 0.3$). On the other hand, it is clear that the peak currents increase with the rising of the scan rate from 10 to 100 mV s^{-1} , while the peak potentials shift positively. It is observed that the peak currents increase linearly with the square root of scan rate, and the linear regression equation can be expressed as $i (\mu\text{A}) = 11.055 v^{1/2} (\text{mV s}^{-1}) - 24.430$ ($R=0.993$) (Fig. 1C), indicating that the oxidation of IPP is under diffusion control.

3.3 Optimization of experimental parameters

In order to fabricate an efficient sensor, the favorable conditions for fabricating the β -CD-rGO sensor (modified electrode) were investigated. Cyclic voltammetric technique was used to optimize the experimental conditions.

3.3.1 Temperature

Temperature has tremendous influence on the peak current of IPP, which is rarely mentioned. It is clear that the peak current increases rapidly when temperature decreases and it achieved the maximum value at 0 $^{\circ}\text{C}$ (Fig.2 B). Because electrolyte is aqueous solution which will be frozen under 0 $^{\circ}\text{C}$, the optimized temperature was chosen to be 0 $^{\circ}\text{C}$. In order to understand the cause of current increase, CVs of GCE (Fig.2 A) and rGO/GCE (Fig.2 C) with 50 μM IPP in 0.1 M PBS

(pH=7.0) at different temperatures were investigated. There are two peaks (around -0.6 V and -1.1 V) on three GCEs, in accordance with two peaks in HPLC chromatogram which means there are two isomers of IPP. The current of peak at -1.1 V increases rapidly when temperature decreases while the current of peak at -0.6 V decreases slowly when temperature decreases. Besides, the peak at -1.1V is more narrow and current is much higher, which is beneficial for quantitative detection of IPP. As the peak current and potential on bare GCE change with temperature like β -CD-rGO/GCE and rGO/GCE, it is assumed that the two isomers are affected by temperature and the modified electrodes amplified the electrochemical signals. In order to confirm the isomers of IPP, high-performance liquid chromatography of IPP in PBS (pH=7.2) was exhibited in Fig. 3. The two well separated peaks provide the evidence of the two IPP isomers. To further clarify the interaction of IPP and β -CD-rGO/GCE, we employed another pesticide methyl parathion (MP), which has similar structure with IPP, as guest molecule for electrochemical detection. It is found that peak current of MP increases a little as temperature decreases. In the study of electrochemical behavior of imidacloprid (IDP)- β -cyclodextrin polymer (β -CDP) [27], it is reported that peak current of IDP- β -CDP increased with temperature which is in accordance with the peak current of IDP on β -CD-rGO/GCE. In this way, IDP will not disturb the detection of IPP. Accordingly, the structure transformation of IPP at low temperature is beneficial to further improve the sensitivity and selectivity of detection .

3.3.2 Accumulation time

The accumulation time also influences electrochemical response of the β -CD-rGO sensor for IPP since it directly influences the degree of adsorption of IPP on the surface of electrode. Fig. 4 shows the peak current of the β -CD-rGO/GCE after incubating in 0.1 mM IPP for different times

from 0 min to 9 min. It can be observed that the peak current increases sharply during the first 7 minutes. A steady-state current was reached after 7 min, suggesting that the adsorption of IPP has reached a saturation state on the surface of β -CD-rGO/GCE. Therefore, 7 min was chosen as the optimal accumulation time.

3.3.3 Solution pH

The pH has tremendous influence on the reduction of IPP. From the reaction mechanism, protons participate in the reduction reaction of IPP. The CV of IPP on the β -CD-GO/GCE at different pH values are shown in Fig. 5 A. It is clearly found that the reduction peak current and the reduction peak potential are changed with the variation of solution pH. Fig. 5 B shows that the reduction peak current of IPP on the β -CD-GO/GCE increases from pH 6.0 to 7.2, and then decreases from pH 7.2 to 7.5. In this case, the optimal pH value is 7.2. The reduction peak potential of IPP on the β -CD-GO/GCE versus the value of pH is shown in Fig. 5 C, in which a straight line with correlation coefficient of 0.999 is obtained. It indicates that protons participate in the reduction of IPP. According to the Nernst equation the relation of between E_p and pH is shown in Eq. (2).

$$E_p = -\frac{0.0592m}{n} pH + b \quad (2)$$

where n is the electron transfer number in the electrochemical reaction, m is the number of protons participating in the electrochemical reaction. From the straight line in Fig. 5 C, the slope of line is -0.055, implying that the value of m is equal to that of n and further confirms the validity of mechanism.

3.4 Binding affinity experiments

In order to investigate the influence of temperature on peak current improvement of IPP on

β -CD-rGO/GCE, the binding affinity experiments of IPP guest to the β -CD-rGO host was studied.

The influence of increasing concentrations of IPP on their reduction peak currents at 0 °C and 25 °C was investigated by LSV in PBS. The association constant (K) of IPP with β -CD on the electrode surfaces can be deduced using the following equation derived from Langmuir isotherm (Eq. (3)) [28].

$$\frac{[IPP]}{I_{pc}} = \frac{1}{KI_{max}} + \frac{[IPP]}{I_{max}} \quad (3)$$

Where I_{pc} is the reduction peak current for a given concentration of IPP, I_{max} is the maximum peak current and K is the binding constant of IPP with β -CD. As shown in Fig. 6, the plots of reciprocal of peak current ($1/I_{pc}$) vs. reciprocal of concentration ($1/[IPP]$) present two cross straight lines, which means the formation of two inclusion complex in different molar ratio [29].

The K_1 value was calculated to be $1.5 \times 10^4 \text{ M}^{-1}$ at 0 °C from the intercept and slope of the linear equation $1/I_{pc} = 2.4 \times 10^{-4} \times 1/[IPP] + 3.6$ ($R=0.994$) at high concentration of IPP. At the same time, the K_2 value was calculated to be $3.2 \times 10^5 \text{ M}^{-1}$ from the linear equation $1/I_{pc} = 1.1 \times 10^{-4} \times 1/[IPP] + 35.6$ ($R=0.999$) at low concentration of IPP. At 25 °C, K_1 and K_2 were calculated to be $1.0 \times 10^4 \text{ M}^{-1}$ and $5.8 \times 10^4 \text{ M}^{-1}$ according to the equation $1/I_{pc} = 4.8 \times 10^{-4} \times 1/[IPP] + 4.8$ ($R=0.999$) and $1/I_{pc} = 3.3 \times 10^{-4} \times 1/[IPP] + 19.4$ ($R=0.999$), respectively. It is apparent that the binding constant of IPP with β -CD at 0 °C is extremely higher than 25 °C, which provides evidence of improved electrochemical response at low incubation temperature. The results are extremely higher than those of other guest molecules (such as 1960 M^{-1} for levodopa and 1558 M^{-1} for carbidopa [30], 2823 M^{-1} for 1,3-dinitrobenzene [20]) binding with the β -CD host, indicating β -CD immobilized on the β -CD-rGO/GCE surface has the excellent abilities of inclusion and recognition toward IPP molecular. From the above analysis, it can be concluded that the electrochemical response mainly

comes from the surface-confined IPP molecules which are easily included in the cavity of β -CD attached to the rGO film at low temperature. However, the detailed electrochemical response mechanism between IPP and β -CD-rGO/GCE is not very clear at present and needs further study.

3.5 Calibration curve

The $4e^-$ single peak at around -1.0 V for the reduction of IPP on this modified electrode is considered for the sensing purpose. Peak current increase with the increase in concentrations of IPP in CV experiments (Fig. 7 A and B). The linear relationship between the peak currents and the concentrations was obtained for the concentrations ranging from 1 to 10 μ M after pre-concentration at 0 $^{\circ}$ C, by this way can the sensitivity be improved. In order to obtain wider linear range, the linear relationship for the concentration ranging from 10 to 50 μ M was also obtained through pre-concentration at 25 $^{\circ}$ C. The limit of detection (LOD) was found to be 0.20 μ M and the limit of quantitation (LOQ) was found to be 0.67 μ M which were calculated from the calibration curve by using the equations; $LOD=3s/m$ and $LOQ=10s/m$ where s is the standard deviation of the blank and m is the slope of the calibration curve. Typical linear sweep voltammetry (LSV) response of various concentrations of IPP is higher than CV response, which is shown in Fig. 7 C and D. The LOD and the LOQ were found to be 0.11 μ M and 0.37 μ M, respectively. This analytical performance can be compared with those recently reported in the literature. For example, the LOD of imidacloprid determination using β -CDP-rGO/GCE was 0.1 μ M [31] and Nanosilver Nafion/nanoTiO₂ Nafion/GCE was 0.25 μ M [12]. A renewable silver amalgam film electrode was developed for the determination of clothianid and dinotefuran with LOD of 0.20 and 0.67 μ M, respectively [13]. Enhanced amperometric sensing for nitenpyram detection was designed based on the synergistic effect of copper nanoparticles and nitrogen-doped

graphene with LOD of 2.0 μM [14]. It is apparent that metal material is the main solution to improve the determination sensitivity of neonicotinoid insecticide, while β -CD-rGO/GCE provides an inexpensive and simple method to detect IPP residue with a low LOD of 0.11 μM , which is beneficial to practical application.

3.6 Reproducibility and stability of β -CD-rGO/GCE

The long-term storage and operational stability of the electrode are essential for the continuous monitoring of IPP. During the test, ten of the β -CD-rGO/GCEs prepared using the same procedure were investigated by comparing the response current of these electrochemical sensors towards 2 μM and 20 μM IPP. The relative standard deviations (RSD) of the peak currents were calculated to be 4.5% and 3.9%, respectively, which revealed that the electrochemical sensor had good reproducibility. The long-term stability was explored by measuring 2 μM and 20 μM IPP solutions intermittently. The results show that the catalytic current response maintains 92.6% of its initial value even after storing the modified electrode at room temperature for one week, reflecting the good stability of the IPP sensor. The presence of interfering species that can be reduced at the negative potential is a major challenge for carrying out the real sample analysis. Lower reduction potential observed for the reduction of IPP on this β -CD-rGO modified electrode eliminates this problem.

3.7 Interference studies

The possible interference of several inorganic species and pesticides on the reduction signal of 10 μM IPP was carried out by using CV (Fig. 8). The concentrations of the inorganic ions and pesticides added were 10 μM . The inorganic ions such as Mg^{2+} , Ca^{2+} , Fe^{2+} , K^+ , Na^+ , and NH_4^+ did not influence the IPP reduction signal. Possible interference with the other nitroaromatic pesticides

such as methyl parathion, parathion and fenitrothion was also analyzed. These pesticides are reduced at around -0.45 V, whereas the reduction potential of IPP is around -1.0V. The wide variation in these peak potentials makes the β -CD-rGO sensor suitable for sensing of IPP even in the presence of other nitro pesticides.

3.8 Practical applications

The analytical utility of the above modified electrode was checked with three kinds of grain samples. The grains were purchased from the local market and it was crushed and tested for the presence of IPP. The analyzed sample contained no IPP. So five levels of standard IPP concentration was spiked into the 10g of sample and left for one day. Then it was prepared as 2.6 before electrochemical and HPLC analysis. The samples spiked by 1.6, 6.4, 8.0, 10.0 μ M IPP were detected by LSV after accumulation at 0°C for 7 min, while 10.0 and 32.0 μ M IPP spiked samples were detected after accumulation at 25°C. The recovery obtained from the electroanalytical techniques are summarized in [Table 2](#). HPLC analysis of IPP is also carried out. The results obtained in electroanalytical techniques have good agreement with the HPLC method.

4 Conclusions

A novel CD-rGO modified electrode was prepared for the electroanalysis of IPP. The electrode shows much better electrocatalytic activity towards the reduction of IPP which is evident from the lower reduction potential and high sensing current. Low temperature is applied to further improve the determination sensitivity as a simple solution. It can be easily deployed for food analysis. The recovery rate of the standard was tested in samples. The results show good agreement with the HPLC method. The proposed sensor is thus opened a new opportunities for the sensing of new neonicotinoid insecticide IPP in the food samples.

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Table 1 Linear range, variation coefficient, linear equation, LOD and LOQ of IPP.

Electrochemical method	Temperature	Linear range (μM)	Linear equation	Variation coefficient	LOD (μM)	LOQ (μM)
CV	0 °C	1-10	$I=1.66C+1.68$	0.9974	0.20	0.67
	25 °C	10-55	$I=0.44C+2.87$	0.9978		
LSV	0 °C	1-10	$I=3.27C+3.71$	0.9949	0.11	0.37
	25 °C	10-55	$I=1.32C+6.27$	0.9984		

Table 2. Recovery studies of standard IPP spiked grain sample at β -CD-rGO/GCE.

Sample	Temperat	Amount	Amount	Recovery	Content	Content
	ure	added	recovered	rate	determined	determined
		(μ M)	(μ M) $\pm$ SD	(%)	by LSV	by HPLC
					(mg kg ⁻¹) $\pm$ SD	(mg kg ⁻¹) $\pm$ SD
Wheat	0°C	1.6	1.48 $\pm$ 0.05	92.56	0.43 $\pm$ 0.01	0.46 $\pm$ 0.02
		6.4	5.97 $\pm$ 0.13	93.22	1.75 $\pm$ 0.04	1.82 $\pm$ 0.04
		8	7.72 $\pm$ 0.23	96.45	2.26 $\pm$ 0.07	2.32 $\pm$ 0.01
		10	9.83 $\pm$ 0.19	98.35	2.88 $\pm$ 0.06	2.82 $\pm$ 0.09
	25°C	10	9.34 $\pm$ 0.24	93.41	2.74 $\pm$ 0.07	2.82 $\pm$ 0.09
		32	28.88 $\pm$ 0.39	90.26	8.47 $\pm$ 0.11	8.98 $\pm$ 0.04
Millet	0°C	1.6	1.51 $\pm$ 0.03	94.42	0.44 $\pm$ 0.01	0.46 $\pm$ 0.02
		6.4	5.91 $\pm$ 0.20	92.27	1.73 $\pm$ 0.06	1.74 $\pm$ 0.05
		8	7.41 $\pm$ 0.34	92.61	2.17 $\pm$ 0.10	2.19 $\pm$ 0.01
		10	9.88 $\pm$ 0.13	98.78	2.90 $\pm$ 0.04	2.80 $\pm$ 0.02
	25°C	10	9.99 $\pm$ 0.26	99.89	2.93 $\pm$ 0.08	2.80 $\pm$ 0.01
		32	29.01 $\pm$ 0.32	90.65	8.51 $\pm$ 0.09	8.91 $\pm$ 0.11
Rice	0°C	1.6	1.53 $\pm$ 0.06	95.47	0.45 $\pm$ 0.02	0.46 $\pm$ 0.01
		6.4	5.80 $\pm$ 0.15	90.55	1.70 $\pm$ 0.04	1.78 $\pm$ 0.02
		8	7.52 $\pm$ 0.21	93.94	2.20 $\pm$ 0.06	2.24 $\pm$ 0.06
		10	9.97 $\pm$ 0.04	99.66	2.92 $\pm$ 0.01	2.76 $\pm$ 0.01
	25°C	10	9.88 $\pm$ 0.02	98.79	2.90 $\pm$ 0.01	2.76 $\pm$ 0.01
		32	29.46 $\pm$ 0.02	92.07	8.64 $\pm$ 0.01	9.03 $\pm$ 0.03

Scheme 1. Schematic procedure for the preparation of β -CD-rGO/GCE and our concept for enhancing the sensitivity of electrochemical IPP detection.

Fig. 1. (A) CVs of bare GCE (a); rGO/GCE (b); β -CD-rGO/GCE (c) with 50 μ M IPP in 0.1 M PBS (pH=7.0) at 0 °C for 10 min. (B) CVs of β -CD-rGO/GCE with 80 μ M IPP in 0.1 M PBS (pH=7.0) at scan rate ranging from 10 to 100 mV s^{-1} . v (mV s^{-1}): a. 10; b. 20; c. 30; d.40; e. 60; f. 80; g. 100. (C) Plots of peak currents vs. square root of scan rates. (D) Plots of reduction potentials vs. logarithm of scan rates.

Fig. 2. CVs of (A) GCE, (B) β -CD-rGO/GCE, (C) rGO/GCE with 50 μM IPP in 0.1 M PBS (pH=7.0) at incubation temperature ranging from 0 to 50 $^{\circ}C$: a. 0; b. 10; c. 20; d.30; e. 40; f. 50. (D) Plots of peak current vs. incubation temperature on β -CD-rGO/GCE and rGO/GCE.

Fig. 3 HPLC chromatogram of 100 μ M IPP in 0.1 M PBS (pH=7.2).

Fig. 4 CVs of β -CD-rGO/GCE with 50 μ M IPP in 0.1 M PBS (pH=7.2) at 0 $^{\circ}$ C for different incubation times ranging from 1 to 9 min: a. 1; b. 2; c. 3; d. 4; e. 5; f. 6; g. 7; h. 8; i. 9.

Fig. 5. (A) CVs of β -CD-rGO/GCE with 50 μ M IPP in 0.1 M PBS at pH value ranging from 6.0 to 7.5: a. 6.0; b. 6.5; c. 6.8; d. 7.0; e. 7.2; f. 7.4; g. 7.5. (B) Plots of peak current vs. pH. (C) Plots of potential vs. pH.

Fig. 6 Plots of reciprocal of peak current vs. reciprocal of concentration. (A) Accumulation at 0 °C for 7 min; (B) Accumulation at 25 °C for 7 min.

Fig. 7. (A) CV and (B) LSV response of different concentrations of IPP: a. 1; b. 2; c. 4; d. 6; e. 8; f. 10 μM on $\beta\text{-CD-rGO/GCE}$ in 0.1 M PBS (pH 7.2) at 0 $^{\circ}\text{C}$ for 7 min. Sweep rate: 50 mV s^{-1} . (C) CV and (D) LSV response of different concentrations of IPP: a. 10; b. 15; c. 25; d. 35; e. 45; f. 55 μM on $\beta\text{-CD-rGO/GCE}$ in 0.1 M PBS (pH 7.2) at 25 $^{\circ}\text{C}$ for 7 min. Sweep rate: 50 mV s^{-1} .

Fig. 8 Influence of various inorganic ions and nitroaromatic pesticides on the reduction of IPP (10 μM).

Author Biographies

Min Zhang is currently a Ph. D. candidate at School of Chemical Engineering and Technology, Harbin Institute of Technology, China. Her PhD thesis adviser is Prof. Xin Yang at Harbin Institute of Technology. She obtained her M.S. degree from Southwest University. Her current scientific interests are focused on food safety and electrochemical analysis.

Haitian Zhao is an associate professor at Harbin Institute of Technology. He obtained his Ph. D. and M.S. degree from Harbin Institute of Technology. He is mainly engaged in the study of food quality and safety.

Xin Yang is a professor and associate director of Department of Food Sciences and Engineering, School of Chemical Engineering and Technology, Harbin Institute of Technology. He is mainly engaged in the study of food quality and safety.

Aijun Dong is an associate professor at Harbin Institute of Technology. She obtained her M.S. degree from Harbin Institute of Technology. She is mainly engaged in the study of analysis technology.

Hua Zhang is a lecturer at Harbin Institute of Technology. She obtained her Ph. D. and M.S. degree from Harbin Institute of Technology and Northeast Agriculture University, respectively. She is mainly engaged in the study of food quality.

Jing Wang is a professor and director of residues research department of the Institute of Quality Standards & Testing Technology for Agro-products, CAAS. She has engaged in studies of food safety and testing technology.

Guangyang Liu is currently a Ph. D. candidate at Harbin Institute of Technology, China. He obtained his M.S. degree from Yantai Institute of Coastal Zone Research Chinese Academy of Sciences. His current scientific interests are focused on nano-detection technology.

Xingchen Zhai is currently a Ph. D. candidate at Harbin Institute of Technology, China. She obtained her B.S. degree from Harbin Institute of Technology. Her current scientific interests are focused on food safety.

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